

DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO FILE

(BNF 000057)

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Keywords

Brassica napus L. *oleifera* varieties, male sterility, barnase and barstar genes, herbicide resistance (glufosinate -ammonium)

Background

In a submission dated May 29, 1998, AgrEvo provided summary information to support their safety assessment of genetically modified canola, specifically the LibertyLink Trait - an oilseed rape containing a new hybrid system MS8/RF3. This data is further supported by documents submitted to FDA in 1995 regarding three transgenic oilseed rape lines: a male sterile oilseed rape line, designated MS1 and two restorer lines designated RF1 and RF2.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification is to be able to produce F1 hybrids. F1 hybrid varieties produce an estimated yield of 20-25% more oil than the best open-pollinated oilseed rape varieties. Oil from the canola seed is used in food and meal made from the seeds is used in animal feed.

According to AgrEvo, their male sterile oilseed rape plants have been developed by introducing a ribonuclease (barnase) gene which is expressed exclusively in the tapetum, the layer of cells surrounding the pollen sac, during anther development. Expression of barnase in this tissue blocks pollen development and results in a male sterile plant, designated MS8 (DBN230-0028). Additionally the barstar gene (ribonuclease inhibitor) has also been introduced into a line of oilseed rape, and was used to develop the restorer line of the hybrid system. This line was designated RF3. Crossing a male sterile and a restorer line results in fertile oilseed rape since the barstar protein will inactivate the barnase enzyme. Both components are linked to a glufosinate-ammonium resistance gene, bar, which allows for selection in both the transformation and regeneration of the lines.

Molecular Alterations

AgrEvo has described the identity and function of the genetic material introduced into the canola lines by the *Agrobacterium tumefaciens* Ti plasmid-mediated transformation system. The genes introduced to produce the male sterile line were contained on the plasmid pTHW107 and those used to produce the restorer line were contained on plasmid pTHW118.

AgrEvo reported the components and origins of the sequences of pTHW107 plasmid. The significant components included 1) the coding sequence of the bialaphos resistance gene (bar) of *Streptomyces hygroscopicus* 2) the promoter from the atS1A ribulose- 1,5-biphosphatecarboxylase small subunit gene from *Arabidopsis thaliana* 3) the coding region of the barnase gene from *Bacillus amyloliquefaciens* and 4) the promoter region of the anther-specific gene TA29 from *Nicotiana tabacum*.

AgrEvo reported the components and origins of the sequences of pTHW118 plasmid. The significant components included 1) the coding sequence of the bialaphos resistance gene (bar) of *Streptomyces hygroscopicus*, 2) the promoter from the atS1A ribulose- 1, 5-biphosphatecarboxylase small subunit gene from *Arabidopsis thaliana*, 3) the coding region of the barstar gene from *Bacillus amyloliquefaciens* and 4) the promoter region of the anther-specific gene TA29 from *Nicotiana tabacum*.

Expressed Protein

The selectable marker gene, bar, encodes the enzyme phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme acts by transferring the acetyl group of Acetyl Co-A to the herbicide glufosinate-ammonium (phosphinothricin) rendering it inactive. PAT is expressed mainly in green tissue. Its levels in seeds were <0.002% of the total extractable protein. No PAT was detected in the canola oil or canola meal.

According to AgrEvo, the barnase and barstar genes code for single chain proteins. Barnase is the common name for a specific extracellular ribonuclease secreted by the bacterium *Bacillus amyloliquefaciens*. Ribonuclease enzymes are naturally occurring and found in many types of organisms, including bacteria and eukaryotes. Barstar is the name for a specific inhibitor of the barnase enzyme. The inhibition of the Barnase enzyme by Barstar is highly specific. Both proteins are relatively small and have been the subject of studies for several years

Compositional Analysis

AgrEvo presented data concerning the oil, erucic acid, glucosinolate, various minerals, and protein content in the seeds of the new variety compared to their non-transgenic and commercial variety counterparts. They also presented information on the fatty acid composition of the oil of the new variety compared to their non-transgenic and commercial variety counterparts. No significant differences in processing characteristics or composition resulting from the insertion of the male sterility/restorer genes were found when compared to their non-transgenic and commercial variety counterparts.

Conclusions

AgrEvo has concluded that the hybrid canola containing LibertyLink Trait made with transformation events MS8/RF3 are not materially different in composition, nutrition, and safety from canola currently grown, processed, marketed, and consumed from human

food and animal feed. At this time, based on AgrEvo's description of its data and analysis, the Agency considers AgrEvo's consultation on the hybrid canola containing the LibertyLink Trait from transformation events MS8/RF3 to be complete.

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